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REPRODUCTION AND MORTALITY OF CALIFORNIA RED SCALES RESISTANT AND NONRESISTANT TO HYDROCYANIC ACID GAS, AS AFFECTED BY TEMPERATURE [1]

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INTRODUCTION

It was first pointed out by Quayle (*5*, *6*)[3] that in different areas the California red scale (*Aonidiella aurantii* (Mask.)), a major pest of citrus and other fruits, varies greatly in susceptibility to hydrocyanic acid gas. Quayle's findings have been corroborated by other workers. Dickson (*2*) and Yust et al. (*10*) have shown that resistance to hydrocyanic acid is an inherited sex-linked character. Yust et al. (*11*) and Lindgren and Dickson (*3*) have also shown that repeated fumigations of different stocks of scales may gradually increase their resistance to hydrocyanic acid. Since most field populations of red scale are probably mixtures of resistant and nonresistant scales, the selective action of repeated fumigations might tend to make the insect more difficult to control, unless biological factors operate to favor the nonresistant scale. An investigation of the comparative biology of the two strains was therefore undertaken at Whittier, Calif. The present paper reports the results of studies on reproduction and mortality of the two strains under different temperatures.

METHODS

Two stocks of scales were originally collected in the field by F. S. Stickney in 1935, the nonresistant strain from Escondido and the resistant strain from Corona, Calif. They were reared in isolated compartments at Whittier from 1935 to 1946, inclusive, with no substantial change in their relative resistance to hydrocyanic acid. In a number of parallel tests with hydrocyanic acid gas on different generations, the mortality of the second-molt [4] scales averaged 99.3 percent for the nonresistant and 32.5 percent for the resistant strain (*10*). Although the relative susceptibility of the two strains varied considerably on different dates, no consistant increase or decrease was

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[2] The author is indebted to A. W. Cressman for advice and suggestions during the course of these studies.

[3] Italic numbers in parentheses refer to Literature Cited, p. 162.

[4] Definitions of stages throughout this paper follow Yust et al. (*8*).

evident. These stocks were used in the first part of the work, but beginning in December 1942, most of the resistant scales were taken from a part of the resistant stock that had been given 14 fumigations in 15 generations (*11*).

The methods of rearing the scales were similar to those described by Yust and Munger (*9*), except that in the mortality studies a half-lemon technique was used, to eliminate variability due to individual fruit differences. A sticky banding material, smeared on a paper ribbon or on a piece of twine, was fastened around the middle of each lemon. Both strains of the scale could then be reared on the same fruit. This method could not be used in the reproduction studies in which records on settling were desired, but fruits were carefully selected for uniformity.

REPRODUCTION

AT CONSTANT TEMPERATURE

The first group of scales was subjected to a constant temperature of 77° F. and 65 percent relative humidity.[5] Individual records on crawler production were kept during the entire life of the females. The individual scales were confined within gelatin capsules, as described by Yust (*7*), but methyl alcohol, instead of ethyl alcohol, was used in the sealing paste. Thirty-two mature scales of each strain were thus confined, but decay of a number of lemons permitted only 22 resistant and 19 nonresistant scales to complete their reproductive period. Inspections were made daily. As shown in the first line of table 1, the resistant scales produced an average of 305.7 live crawlers per female, and the nonresistant scales an average of 326. The difference was not statistically significant.

TABLE 1.—*Reproduction of California red scales under different temperature conditions*

Rearing condition	Duration of experiment	Reproducing females		Scales produced per female	
		Resistant	Nonresistant	Resistant	Nonresistant
		Number	*Number*	*Number*	*Number*
77° F. constant and 65 percent relative humidity.	Entire reproductive period of scales.	22	19	305.7	326.0
Laboratory, high fluctuating temperatures.	7 weeks	95	95	64.5	64.1
Winter lath house	Nov. 6, 1942–Apr. 16, 1943	100	100	135.5	148.5
Summer lath house	July 5–Sept. 9, 1943	60	60	113.1	217.4
Do	July 10–Aug. 23, 1943	60	60	79.2	146.9

AT HIGH FLUCTUATING TEMPERATURES

A second group of scales was subjected to controlled fluctuating temperatures which duplicated thermograph records from Corona, Calif., for the 4-week period July 15 to August 11, 1933. These temperatures were representative of hot, interior California conditions and were reproduced in the laboratory for 7 weeks by means of patterns

[5] The constant-temperature studies were made by B. M. Broadbent.

for the variable thermostat (Munger *4*), first using the 4-week record and then repeating records for weeks 1, 3, and 4. Week 2 was omitted from the second period because it was a week of abnormally high temperatures, 4 days being well above 100° F. The maximum, minimum, and mean daily temperatures for this period are shown in figure 1. Moisture in the chamber was maintained by means of an

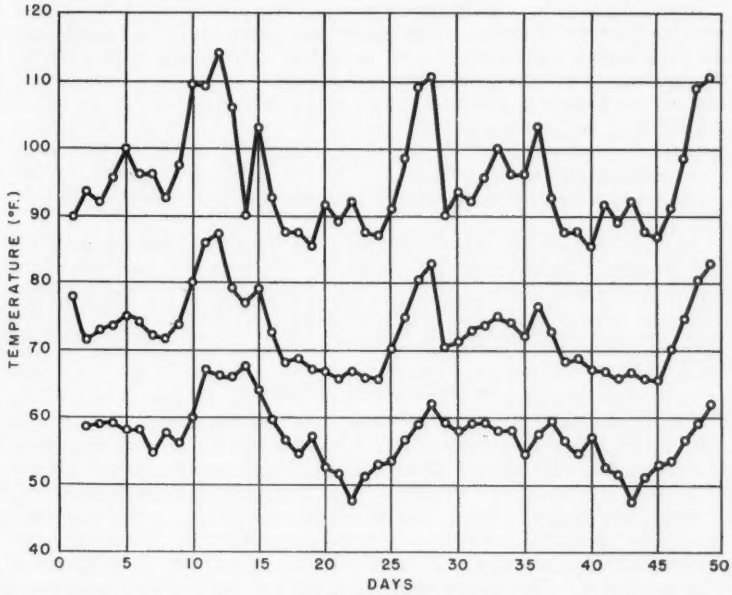

FIGURE 1.—Maximum, mean, and minimum daily temperatures of the 7-week program in which Corona, Calif., temperatures were reproduced in the laboratory.

open pan filled with water, over which air was continuously forced by means of a blower.

On November 12, 1943, 50 lemons were lightly infested, 25 with resistant and 25 with nonresistant scales. The scales were held at 77° F. until the beginning of the 7-week fluctuating-temperature program. As the scales approached the reproducing stage all but 5 mature females were removed from the lemons. On December 29 the experiment was begun. At that time a few crawlers could be seen under the covers of some of the females, but no whitecap stages were observed on the lemons.

The young scales were removed and records were made at weekly intervals. At the end of 7 weeks, 19 of the lemons in each group were in good condition. The results of this experiment, based on the counts from the 38 good lemons, are shown in the second line of table 1. On these lemons 74 resistant and 63 nonresistant scales remained alive. At the end of the first to sixth counts, inclusive, the totals of living resistant and nonresistant reproducing scales were 95 and 95, 89 and 89, 83 and 79, 80 and 73, 79 and 69, and 77 and 69, respec-

tively. The difference between the reproduction of the 2 strains, 0.4 scale per female (table 1), was not statistically significant.

IN THE WINTER LATH HOUSE

Three groups of scales were studied under various lath-house conditions. On September 16, 1942, 2 groups of 33 lemons each were infested lightly, one with the resistant and the other with the nonresistant strain of scale. The scales were held at 77° F. until reproduction began, and then all but 5 mature female scales and the few young that had been produced were removed from each lemon. The scales were placed in the lath house on November 6, 1942. The young scales were removed and counted at intervals of 11 to 22 days between November 19, 1942, and April 16, 1943. During this period the temperature ranged from 37° to 90°. Each of these extremes occurred but once, and on only 4 days did the temperature fall below 40°.

At the time of the April 16 count, 20 lemons of each group were still in good condition, and on them 86 resistant and 88 nonresistant scales were still alive. During the reproduction period measured (table 1) the nonresistant scales produced 13 more young per scale than did the resistant scales, but this difference was not statistically significant.

IN THE SUMMER LATH HOUSE

The third and fourth groups of scales, prior to being placed in the lath house, had been subjected to a 24-day period of fluctuating temperatures, with slowly decreasing minimum temperatures designed to harden the scales to withstand low temperatures in the first- and second-molt stages. From the twentieth to the twenty-second day of the 24-day period, while the scales were mostly in the molting stages, they were subjected to a minimum temperature of 29° F. on three successive nights. The scales that encountered the low temperatures in the second-molt stage were in the lath house from July 5 to September 9, 1943. The ones that encountered the low temperatures in the first-molt stage were in the lath house from July 10 to August 23, 1943. In both these summer-lath-house tests the nonresistant scales produced considerably more young than the resistant scales. This greater production, however, was linked with a higher survival of the nonresistant females.

Such a difference in productivity would be of considerable importance if it were consistent, and therefore a number of similar experiments were made. Since the mortality of the reproducing females, rather than the production per living female, was shown to be the important factor in determining the number of young, future data were limited to the mortality during the reproducing period, thus permitting the use of a much larger number of scales. The scales were exposed to low temperatures, as in previous tests, and were then held at 77° F. until reproduction began, after which they were placed in the lath house. Results of additional tests are given in table 2.

Only 1 of the 4 groups (first molt, observed from July 31 to Sept. 11, 1944) showed any great difference in mortality. This group was

TABLE 2.—*Mortality of reproducing California red scales in the lath house at Whittier, Calif.*

Stage of scale exposed to low temperature	Scales of each strain	Reproductive period observed	Mortality of scales	
			Resistant	Nonresistant
	Number		*Percent*	*Percent*
First molt	600	Aug. 6–Oct. 19, 1943	58.0	60.0
Second molt	1,200	Aug. 6–Sept. 20, 1943	9.6	4.8
Do	1,200	July 22–Aug. 25, 1944	5.9	5.8
First molt	600	July 31–Sept. 11, 1944	52.8	19.2

exposed to rather high temperatures on 2 days (maxima, 105° and 103° F.) shortly before the mortality counts were made, in contrast to the other groups, which had encountered no high temperatures. Since it was possible that the combination of low temperatures in the molting stages and high temperatures during the reproductive period had caused an abnormal mortality of the resistant scales, tests were made, as follows: After the scales on 12 lemons had been exposed to low temperatures in the first-molt stage, they were held at 77° until reproduction began, and then 6 of the lemons were subjected to alternate weekly hot and cool temperatures from November 23 to December 27, 1944. The daily maximum in the hot week ranged from 90° to 105°, and the maximum in the cool week was 80°. The other 6 lemons were kept at 77°. Scales on a second lot of 12 lemons were not exposed to low temperatures in the first molt, but were reared at 77° until reproduction began, when they were divided, as was the first lot. The highest mortality in any of these groups was 23 percent, and there was no difference between the 2 strains. Tests with a large number of female scales have therefore failed to show any consistent difference in mortality which would lead to differences in the number of young produced.

Although the factors responsible for the three instances of differences in the mortality of the reproductive stage after earlier conditioning to low temperatures have not been isolated, it does not seem likely that the occasional occurrence of these differences indicates any important biological advantage for the nonresistant strain. The conditioning of the first- and second-molt stages required a fall in temperature much more gradual than is common in nature. Very few of the first- and second-molt stages survive winter temperatures in the field.

MORTALITY

Records on mortality were obtained in a lath house on the laboratory grounds at Whittier, Calif., in different seasons and also under various controlled fluctuating temperatures.

IN THE LATH HOUSE

Scales were reared at 77° F. until they reached the desired stages. Equal numbers of scales of each strain were then marked on each lemon and the fruit was placed in a lath house to determine the effect of outdoor conditions on mortality. The results are summarized in table 3.

TABLE 3.—*Mortality of California red scales in the lath house, Whittier, Calif.*[1]

Period in lath house	Stage of scale placed in lath house	Scales of each strain	Mortality of scales	
			Resistant	Nonresistant
		Number	*Percent*	*Percent*
Nov. 21, 1941–Mar. 5, 1942	Whitecap	300	97.0	96.0
Feb. 17–Apr. 21, 1942	do	1,200	75.2	72.7
Nov. 18, 1942–Apr. 16, 1943	do	600	95.3	92.2
Mar. 2–18–June 3–15, 1943	do	2,160	75.6	62.3
Apr. 3–June 18, 1943	do	600	58.0	50.8
June 26–27–Aug. 24–28, 1943	do	2,400	43.8	61.9
Nov. 12, 1942–Apr. 2, 1943	First molt	300	91.7	84.3
Dec. 5, 1941–Mar. 27, 1942	Second stage	90	78.8	81.1
Nov. 28, 1942–Apr. 14, 1943	do	600	63.5	77.8
Dec. 5, 1941–Mar. 27, 1942	Second molt	90	73.3	78.8
Nov. 25, 1942–Apr. 13, 1943	do	360	81.7	84.1
Dec. 19, 1941–Mar. 30, 1942	Gray adult	30	36.6	23.0
Dec. 7, 1942–Apr. 17, 1943	do	300	65.7	63.0
Jan. 2, 1942–Mar. 30, 1942	Mature	120	44.2	10.0
Nov. 10–23, 1942–Mar. 3–Apr. 1, 1943	do	1,800	14.8	14.7

[1] Some groups include scales that settled and were put in the lath house on different days.

There seemed to be no consistent differences in mortality between the two strains. In four groups the differences in mortality were statistically significant when each group was analyzed separately. In two groups (March 2–18—June 3–15, 1943, and November 12, 1942—April 2, 1943) the resistant strain had the greater mortality; in two (June 26–27—August 24–28, 1943, and November 28, 1942—April 14, 1943) the mortality was greater in the nonresistant strain.

Whitecap scales kept in the lath house through the winter months suffered very high mortality—96.5 percent in 1941–42 and 93.8 percent in 1942–43. Most of the surviving scales were mature when the final mortality counts were taken, but records were made of the stages in which death occurred. The molting stages were the most critical. During the months of March to June, inclusive, only whitecaps were put in the lath house, but the surviving scales had reached the mature stage by the time final records were made. The mortality of such groups as are recorded in table 3, therefore, is a summation of scales dying in all stages.

The lowest temperatures for the winter of 1941–42 were in December, January, February, and March, with minima of 37°, 35°, 33°, and 38° F., respectively. In the same months for the winter of 1942–43 they were 38°, 39°, 37°, and 44°, respectively.

AT HIGH FLUCTUATING TEMPERATURES

In tests with a variable-temperature thermostat, scales were subjected to artificially fluctuating temperatures and to selected summer- and winter-temperature patterns taken from thermograph records at Corona, Calif. The Corona records were selected as representative of interior conditions in a region where the red scale is a severe pest. Earlier studies by Munger[6] had shown that the body temperature of scales in the shade corresponds closely to the air temperature, whereas that of scales exposed to the sun under California conditions may be

[6] MUNGER, F. BODY TEMPERATURE MEASUREMENTS OF THE CALIFORNIA RED SCALE. Unpublished manuscript.

more than 21° above the air temperature. Some of the present tests were based on scale temperatures in the sun, but most of them were based on shade temperatures, since the bulk of the normal scale population is in the shade. Prior and subsequent to the fluctuating-temperature tests, the scales were reared at 77° F. Mortality counts were made about 3 weeks after the scales were removed from the fluctuating-temperature cabinet.

A high-temperature program consisted of a reproduction of the Corona thermograph record for the week ending July 28, 1933. This record was chosen primarily because the day temperatures were high. The maximum daily temperatures for this week were as follows: 92.5°, 97°, 99°, 99°, 113°, 106°, and 90° F. The lowest temperature was 56° on the first night. Scales of all stages (see table 4) were used in

TABLE 4.—*Mortality of California red scales in a fluctuating-temperature cabinet in which the high temperatures of Corona, Calif., were reproduced*

Stage of scale	Scales of each strain	Mortality of scales		Stage of scale	Scales of each strain	Mortality of scales	
		Resistant	Nonresistant			Resistant	Nonresistant
	Number	*Percent*	*Percent*		*Number*	*Percent*	*Percent*
First stage	600	21.3	19.0	Second molt	1,200	27.4	35.2
First molt	900	34.2	32.7	Gray	360	2.8	3.3
Second stage	240	1.3	.8	Mature	1,570	9.9	9.6

this week-long program. In the first and second molts and in the mature scales, results with several groups of scales have been averaged. There was no consistent difference in mortality between the strains. The molting stages and the first stage suffered higher mortality than the second, the gray, or the mature stages. A group of 4,000 scales, reared continuously at 77° from the time of settling to the beginning of reproduction, suffered a total mortality of 28.6 percent, with no differences between the strains.

Since the temperature of scales and of fruit surfaces in the sun may rise well above air temperature, an attempt was made to measure the effect of high temperatures, such as might be encountered occasionally by exposed scales. The temperature pattern followed was based in part on thermograph records covering a period of hot weather from September 6 to 9, 1937. During this period the daily temperature ranges were 52° to 98°, 59° to 106°, and 62.5° to 108.5° F., with a final maximum of 110°. The maximum temperatures were corrected by adding the differences between fruit and air temperatures, found by means of thermocouples. The highest differential between air and fruit temperature was 15.2°, which was found when the air temperature registered 106.3°. The complete sequence of minimum and maximum temperatures used in tests of scales of all stages consisted of the following: 52°–109°, 58°–115°, 59°–119.5°, 62.5°–121.5°, 59°–119.5°, 58°–115°, and 52°–109°. The complete test killed all scales and burned the lemons. In tests in which only the first 3 and first 4 days (except for the fourth minimum) were used, some of the scales survived (see table 5).

TABLE 5.—*Mortality of California red scales exposed to fluctuating high temperatures based on temperatures of fruits in the sun when air temperatures are high*

Stage of scale	Mortality of scales exposed first 3 days		Mortality of scales exposed first 4 days	
	Resistant	Nonresistant	Resistant	Nonresistant
	Percent	*Percent*	*Percent*	*Percent*
Whitecap	48.5	47.8	96.7	93.8
First molt	92.7	92.7	99.3	98.0
Second stage	45.6	45.6	92.0	92.0
Second molt	99.2	100.0	100.0	100.0
Gray stage	90.7	92.0	98.0	98.7
Mature	100.0	100.0	100.0	100.0

Although there was practically no difference in mortality between the strains, the whitecap and second stages were the least susceptible to high temperatures.

AT LOW FLUCTUATING TEMPERATURES

Scales were next exposed to lower temperatures, according to a program based in part on the thermograph record of the last week in October 1935 at Corona. It ranged from 55°–94°, 45°–90°, 50°–82°, 39°–66°, 35°–64°, and 37°–70° F., and differed from the recorded temperatures mainly in that the minimum temperatures of the last 5 days were 2°, 3°, 2°, 6°, and 4° higher than the field temperatures. Whitecap, second-molt, and gray stages were used simultaneously in the test. Only 6.7 percent of the gray-stage scales were killed, as compared with 94.6 percent of the second-molt ones. There was no difference between the strains in these two stages. The whitecap resistant and nonresistant scales suffered mortalities of 26.2 and 19.7 percent, respectively.

Further tests were made which provided for a more abrupt drop in temperature near the midpoint. The minimum and maximum daily temperatures used were as follows: 57°–93°, 56°–93°, 55°–93°, 30°–90.5°, 30°–61°, 35°–67.5°, and 53°–62° F. Mature scales used in these tests showed an average mortality of only 11 percent, while second-molt scales came within a fraction of 1 percent of being completely destroyed. In another experiment in which an 11-day program was used, with minimum temperatures of 57°, 56°, 55°, 41°, 57°, 56°, 55°, 31°, 31°, 31°, and 31°, first-molt scales suffered an average mortality of 95.5 percent, while mature scales suffered an average mortality of only 14 percent, with no difference between the strains.

The results suggested that the molting stages might be conditioned to withstand low temperatures. To test this possibility two programs were used. In the first, the minimum and maximum temperatures were as follows: 60°–80°, 55°–75°, 50°–73°, 45°–70°, 43°–73°, 41.5°–72°, 42°–57.5°, 34.5°–55°, 34.5°–58°, 34°–59°, 34°–60.5°, 41°–56.5°, 41.5°–71°, 42°–57.5°, 34.5°–55°, 34.5°–58°, 34°–59°, 34°–60.5°, 41°–56.5°, and 41.5°–72° F. Marked scales were in the first- and second-molt stages from the fifteenth to the eighteenth days of the program, when the temperatures reached a minimum of 34°. The mortality of these scales was low, the highest being 10.3 percent in the

resistant scales in the first molt. In both strains the mortality of the second-molt scales was less than 2 percent.

The second low-temperature conditioning program carried out included lower minimum temperatures than the first, and consisted of 24 days in which the daily minimum and maximum temperatures were as follows: 60°–80°, 55°–75°, 50°–73°, 45°–70°, 43°–73°, 41.5°–72°, 42°–57.5°, 34.5°–55°, 34.5°–58°, 34°–59°, 34°–60.5°, 41°–56.5°, 55°–75°, 50°–73°, 45°–70°, 43°–73°, 41.5°–72°, 42°–57.5°,34.5°–55°, 29°–61.5°, 29°–61.5°, 29°–61.5°, 40°–65°, and 50°–70° F.

By the time the 29° F. minimum temperatures occurred, the first- and second-stage scales in the cabinet had progressed to the first- and second-molt stages. The results of this experiment (table 6)

TABLE 6.—*Relation between development of molting scales and their susceptibility to low temperature*

Stage and development	Mortality of scales	
	Resistant	Nonresistant
	Percent	*Percent*
Late first molt	17.0	18.0
Early first molt	11.7	8.7
Late second molt	42.8	42.2
Early second molt	5.0	1.7

showed clearly that the conditioning period had served to protect many of the scales from being killed by the low temperatures, which in previous tests had produced a high mortality. It was also apparent that the stage of development of the molting scales was related to their susceptibility to low temperatures.

DISCUSSION

Conditions to which resistant and nonresistant strains of the California red scale were subjected in these experiments ranged from low temperatures, which killed nearly all the molting stages, to temperatures high enough to injure the host fruit. No consistent differences between the reactions of the two strains were noted. At certain times scales of one strain were more tolerant of adverse temperatures than those of the other strain, but such differences might be reversed in a later generation.

The tests have furnished considerable information on the conditions that affect mortality of the California red scale. The range of higher temperatures up to 113° F. caused mortality averaging as high as 33.4 and 31.3 percent in the first- and second-molt stages, respectively. Eight hundred scales of both strains, reared at a constant temperature of 77° and marked in the second-molt stage, suffered an average mortality of only 2.3 percent up to the time when reproduction began. A temperature of 121.5° caused complete mortality of some stages, but injury to the fruit may have been a contributing factor. Under field conditions only a small proportion of the scales would ever be exposed to such temperatures. The effect of winter temperatures on

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the molting stages is more important, and these experiments showed that a rapid drop in temperature may kill most of the first- and second-molt stages, even though the minimum temperatures remain above freezing. Bliss et al. (*1, p. 1228*) reported that, in their experiments at Whittier, "Practically none of the new scales settling later than October managed to reach the adult stage and reproduce." In the tests here reported, only 3.5 and 6.2 percent of the whitecaps set out in a lath house in November survived the winter. Minimum temperatures at the laboratory were somewhat higher than those recorded in many nearby citrus groves on lower ground. It is probable, therefore, that in the field very few of the scales produced late in the fall survive the winter.

SUMMARY

Reproduction and mortality of two strains of the California red scale (*Aonidiella aurantii* (*Mask.*) one resistant and the other nonresistant to hydrocyanic acid gas, were studied under various temperature conditions.

There was no consistent evidence that reproductive capacity or mortality was linked with susceptibility to hydrocyanic acid fumigation.

High fluctuating temperatures up to a maximum of 113° F. increased the mortality of the second-molt stage by an average of 29 percent over that observed at 77°. In the laboratory a temperature of 121.5° caused complete mortality of some stages, but in nature relatively few scales are exposed to so extreme a temperature.

The molting stages were the ones most susceptible to low temperatures. A sharp drop to 29° F. caused almost complete mortality of scales in the first- and second-molt stages. Mortality was lowest when temperatures were gradually decreased to the same minimum.

The evidence indicates that few of the scales produced late in the fall survive the winter.

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THE PRODUCTION OF ALATE FORMS OF MYZUS PERSICAE ON BRASSICA CAMPESTRIS IN THE GREENHOUSE [1]

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INTRODUCTION

The green peach aphid (*Myzus persicae* (Sulz.)) reduces the yield of potatoes both through direct feeding injury and through the transmission of potato virus diseases. In Maine winged aphids are largely responsible not only for the initial infestation of potato plants early in the season but also for the wide dispersal of the species and for the continued reinfestation of potato plants throughout much of the summer.

Shands, Bronson, and Simpson (*4*) [3] reported that wild rutabaga (*Brassica campestris* L.) growing in the northeastern part of Maine was an important secondary host on which relatively large numbers of winged, or alate, forms of this aphid developed. In 1942 the present experiment was conducted at Presque Isle to secure information bearing upon the probable importance of this common weed as a host for alate forms of the green peach aphid. The two experimental variables were stage of maturity at which the plants were infested and size of the population with which they were infested.

METHODS OF EXPERIMENTATION

Six-inch flowerpots were used for all plants. Planting dates were so spaced that, when the experiment began, plants in four stages of growth were available, namely, very young, young, early-flower, and mature. Each pot had a single plant when the experiment began except the pots that contained the very young plants, which had four. Soon after colonization these were thinned to a single plant per pot.

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[2] The authors acknowledge the assistance of Pauline M. Stuart, James M. Williams, and Mearl McLaughlin in securing the data on which these results are based.

[3] Italic numbers in parentheses refer to Literature Cited, p. 173.

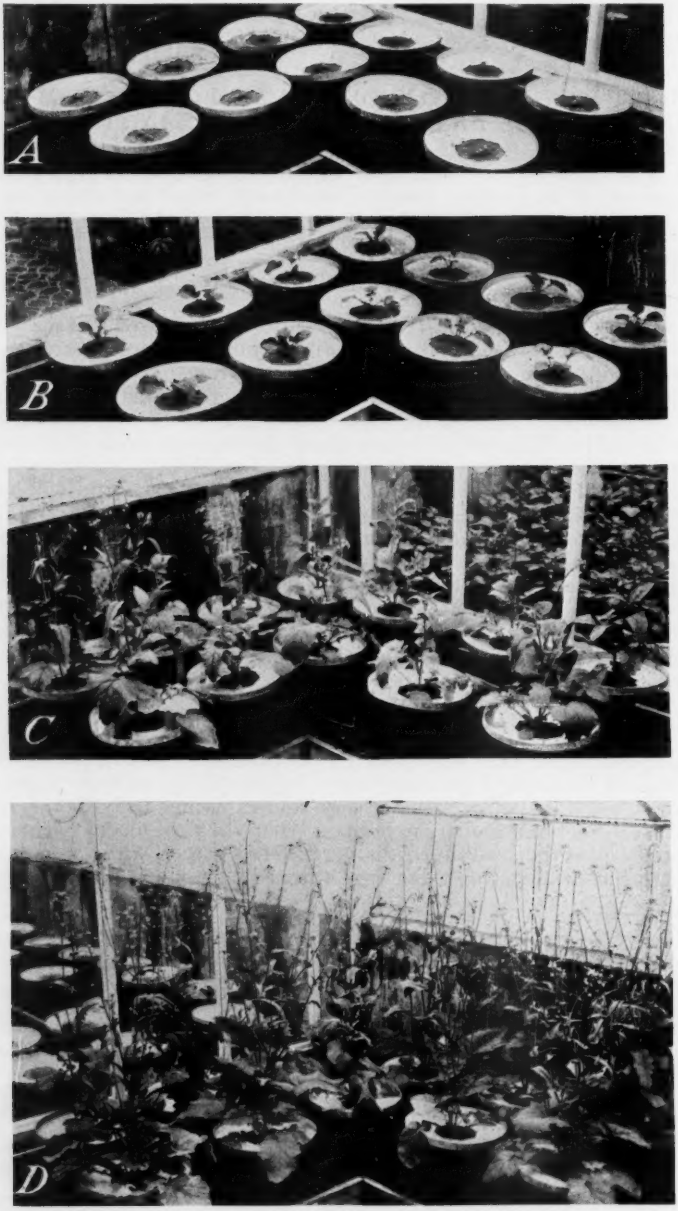

FIGURE 1.—Plants of *Brassica campestris* when the experiment began: *A*. Very young plants (two-leaf stage); *B*, young plants; *C*, early-flower plants; and *D*, mature plants.

Figure 1 shows typical plants in each stage of growth at the beginning of the experiment. The very young plants were in the seedling stage, and the mature plants, although still flowering, had a few seed pods which were practically mature but still green.

Three sizes of aphid population were employed for infesting plants of each stage of maturity; namely, 1, 10, and 100 apterous individuals per plant. There were thus 12 treatments with 8 replications each, arranged in the form of a randomized block. The replicates were divided equally between two similar, adjoining rooms in the greenhouse.

The mature, apterous aphids that were used to infest the plants had been reared in the greenhouse, principally on wild rutabaga plants. All the plants to be used in the experiment were fumigated with a

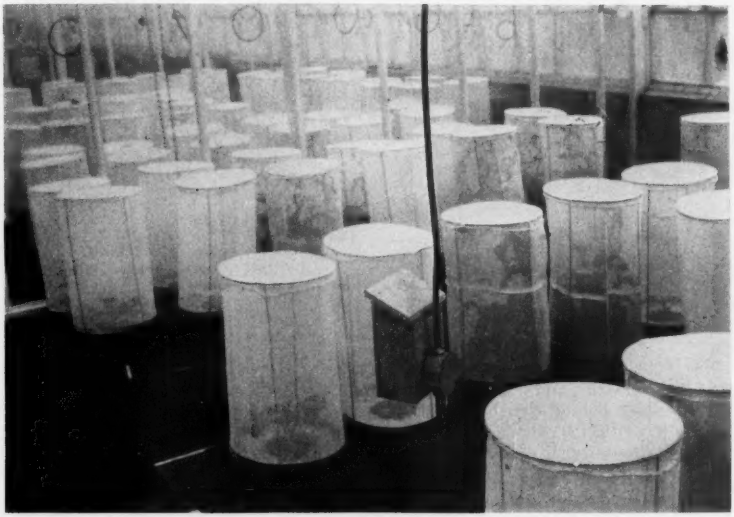

FIGURE 2.—Caged plants of *Brassica campestris*.

nicotine smoke on May 26, and between May 27 and 29 aphids were transferred to the test plants. Selection of the mature apterous individuals was made with the aid of a binocular microscope just before the plant was to be colonized, and immediately after colonization the plant was covered with a cage. Except for short periods of examination for removal of alate forms, each plant remained under its cage until death.

A circular metal pan (fig. 1) with a 4¾-inch opening in the center rested on the top of each pot and held the cage securely over the plant. The cylindrical cage, approximately 13 inches in diameter and 19 inches tall, was covered with tobacco-plant-bed cloth having 32 by 28 threads per square inch. Figure 2 is a general view of some of the caged plants in two greenhouse rooms.

Throughout the experiment, except for 2 days of unusually hot weather when the plants were watered twice a day, the daily watering consisted of 130 to 135 cc. of ordinary tap water poured at one time into the saucer of each pot.

On June 12, when appreciable numbers of alate forms were first seen in the cages, each plant was carefully examined for alate forms of the aphid, and these examinations continued at intervals of 3 to 6 days until the plants died. All specimens found were removed by suction and preserved for later counting. The facilities available did not permit a determination of the number of apterous individuals per plant at any time after colonization.

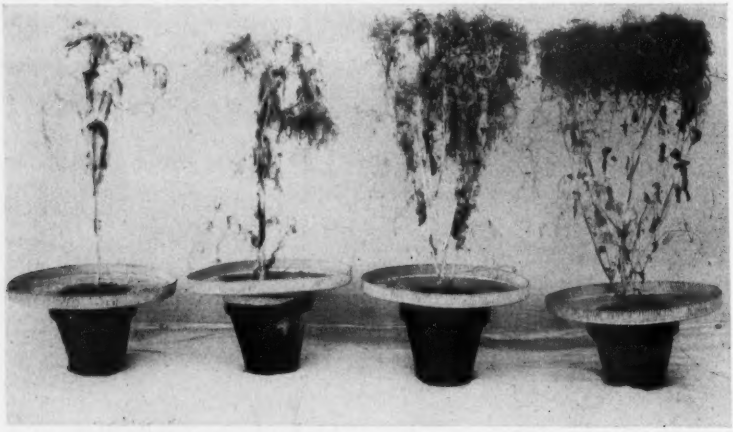

FIGURE 3.—Dead plants of *Brassica campestris* at the close of the study. From left to right, stages of growth are very young, young, early-flower, and mature.

EXPERIMENTAL RESULTS

By August 15 most of the plants were dead, but a few lived until the end of the month. Table 1 shows the total number of alate forms recovered from each plant and the average per plant of each subclass. A study of the data by analysis of variance disclosed that the number of alate forms recovered from very young plants was significantly smaller than the number recovered from plants in other stages of growth, irrespective of the size of the initial population. Very young plants were too small to support the initial populations employed; consequently many of the little plants soon died. Others, while not actually killed by the aphids, were soon in such poor condition that the population was greatly reduced. In such instances the plants recovered before the population increased to any extent, but populations never attained a high level and relatively few alate forms were produced. Figure 3 shows the typical appearance of dead plants at the conclusion of the experiment.

TABLE 1.—*Alate forms of Myzus persicae recovered from caged plants of wild rutabaga growing in the greenhouse when size of initial population and stage of plant growth were variables*

Initial number of apterous individuals per plant	Plant No.	Number of alate individuals recovered from—			
		Very young plants	Young plants	Early-flower plants	Mature plants
1	1	114	2,173	1,972	2,118
	2	413	2,804	2,260	3,088
	3	49	3,250	1,201	3,483
	4	496	1,727	3,797	3,769
	5	1,658	278	1,993	2,363
	6	105	3,415	1,642	703
	7	1,345	2,832	1,231	2,085
	8	93	3,660	2,184	1,253
Average		534.1	2,517.4	2,035.0	2,357.8
10	1	182	3,445	2,340	1,222
	2	232	5,363	3,634	2,944
	3	106	2,262	2,526	1,393
	4	80	3,194	3,766	611
	5	256	3,485	2,162	2,141
	6	99	6,174	2,056	2,327
	7	236	1,783	1,704	2,298
	8	165	2,101	2,070	2,048
Average		169.5	3,475.9	2,532.2	1,873.0
100	1	657	4,486	3,051	1,843
	2	24	583	4,037	3,453
	3	169	1,199	1,138	1,679
	4	26	299	3,719	754
	5	65	2,335	3,163	1,691
	6	16	1,910	1,573	1,674
	7	0	4,250	5,566	3,197
	8	26	1,137	1,961	2,769
Average		122.9	2,024.9	3,026.0	2,132.5

Differences between means of subclasses required for significance (P=0.05) 1018 and (P=0.01) 1355.

Among the other 3 age groups infested with 1 aphid per plant the differences in number of alate forms recovered were not significant (table 1). However, when 10 aphids per plant were employed, the number of alate forms recovered from young plants was almost significantly greater than that recovered from plants in the early-flower stage, and the increase over that from mature plants was highly significant. With an initial population of 100 apterous individuals per plant, the number of alate individuals recovered from early-flower plants approached significance over that from young plants.

When plants of the same stage of growth at colonization but differing in the size of the initial population are considered (table 1), it is apparent that for young plants the number of alate forms recovered from an initial population of 10 apterous individuals per plant was almost significantly greater than that from a colony of 1 per plant, and the increase over that from a colony of 100 per plant was highly significant. For early-flower plants the number of alate forms from a colony of 100 per plant was almost significantly greater than that from a colony of 1 per plant. Differences were not significant for mature plants with different sizes of initial population.

A study of the data showing the number of alate forms recovered at each period of observation suggested that differences might have existed among subclasses in the time and rate at which winged forms

matured. Figure 4 shows the average number of alate forms per plant per day recovered from young, early-flower, and mature plants for each size of initial population. The data for constructing the graphs were computed by dividing the average number of alate forms per plant of each subclass recovered on a given date by the number of days since the previous observation. An arbitrary time of 4½ days was used in deriving the first point on all curves, since it seemed unlikely that many alate forms could have matured under the existing conditions in less than 10 days after colonization.

Figure 4 shows that, in general, production of alate forms began first on plants having the largest initial population, followed in order

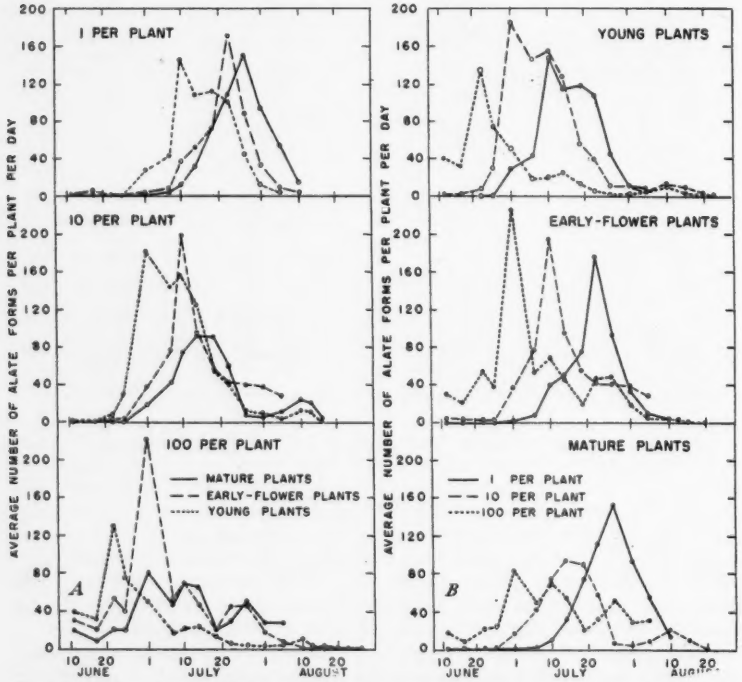

FIGURE 4.—Average number of alate forms of *Myzus persicae* recovered per plant per day from young, early-flower, and mature plants when initial infestation was, respectively, 1, 10, and 100 apterous individuals per plant. *A*, Data grouped according to size of initial population; *B*, according to stage of maturity of plant at time of infestation.

by those having smaller initial populations, and that production of alate forms began earlier with a decrease in plant size at colonization. The time of peak production of alate forms followed similar trends.

DISCUSSION AND INTERPRETATION OF RESULTS

The exact nature of the factors responsible for the results obtained in this study is not entirely clear. Other investigators have found several environmental factors that influence the production of winged

aphids, such, for instance as light, temperature, crowding, starvation, wilting of the host plant, parentage, and the use of certain chemicals in watering the host plant. Ewing (*2*) has reviewed these factors as reported up to 1926. Wadley (*9*), in work with *Rhopalosiphum prunifoliae* (Fitch), found that nutrition, as well as parentage and temperature, was an important factor. Davidson (*1*) reported that winged migrants of *Aphis rumicis L.* developed to some extent as a result of an inherent established tendency, but that the actual number that occurred in any given generation was influenced by overcrowding and the condition of the food plant. He also found that parentage was a significant factor because alate dispersal forms are usually produced by apterous females, and their occurrence in any given generation is affected by overcrowding, nutrition, and temperature. Under suitable conditions of temperature and light, alienicolae generations may be maintained for long periods.

From studies with the mealy plum aphid (*Hyalopterus pruni* (Geoff.)), Smith (*8*) found that the number of drops of excrement produced by individuals per hour decreased as temperature increased. This would indicate that fluid intake was greater at lower temperatures, or that evaporation from the body of the insect was greater at higher temperatures. Smith found a high positive correlation between mean temperature 4 days before birth and the percentage of alate forms produced. He also found that the number of nymphs deposited per day by individuals decreased as temperature increased, and suggested that the fundamental effect of temperature in these findings might be translated into terms of starvation.

Schaefer (*3*) found that a higher percentage of the progeny of the pea aphid (*Macrosiphum pisi* (Kltb.)) became alate when apterous adults were confined to wilted plants than when nymphs in the fourth instar were similarly treated. Like results were obtained when adults and fourth-instar nymphs were starved for 12 or 24 hours and then returned to succulent plants for 46 hours. Schaefer's studies indicated that the mechanism governing wing production is influenced by the concentration of waste products in the body of the mother aphid during the period of wing determination. These waste products were in the form of proteins and carbohydrates, and their accumulation was thought to initiate wing development. The concentration of body contents, hence of waste products, increased when the body fluid was insufficient to eliminate the waste products.

Shull (*6*) found that rudiments of wings were present during the embryonic development of all specimens of the potato aphid (*Macrosiphum solanifolii* (Ashm.)), regardless of whether embryos were destined to become alate or apterous adults. These rudiments could be seen in first-instar nymphs, even in those destined to become wingless adults. His earlier studies (*5*) showed that wing determination occurs within the last 16 to 34 hours before the young aphid is deposited, and his later investigations (*6*) showed that wing differentiation begins about 10 to 12 hours after wing determination. The latter period varied with the changes in light and temperature to which the mother aphid was subjected. Shull's subsequent studies (*7*) indicated that light and heat control wing production through the same mechanism, and he suggested that if a wing-suppressive substance is present it is produced when the mother aphid is subjected to high temperatures, continuous light, or a combination of the two factors.

In the present study several factors may have operated to influence the results (table 1 and figure 4). The more important of these appear to be plant size, density of aphid population, and plant condition. Although the populations of apterous individuals were not determined after colonization, no outstanding differences between subclasses were observed in ratios of alate to total aphid population per plant. Other factors, such as temperature and light, probably had no direct effect, but they may have had an indirect effect by accentuating differences in the physiological condition of plants and insects arising from the action of the principal factors.

Undoubtedly there were large differences in the food supply per aphid under the conditions that prevailed during the experiment. Marked differences, both in the size of plants of each group (figs. 1 and 3) and in the number of aphids placed on the plants, resulted in large differences in area of feeding surface and in the quantity of food available to each aphid. In some instances, at least, these differences were accentuated as the study progressed. Operating singly or together, these factors may have affected the quantities of plant fluid ingested and waste products excreted by the aphids and thus have influenced the number of alate aphids that developed.

Differences in plant condition could have resulted also from the fact that equal amounts of water were supplied to all plants regardless of their size or the density of the aphid population. On the hotter days some of the larger plants became semiwilted, and this may have affected existing nutritional differences arising from other factors.

SUMMARY

An experiment was conducted in the greenhouse to determine the importance of wild rutabaga (*Brassica campestris* L.) as a host of the green peach aphid (*Myzus persicae* (Sulz.)). The study was concerned primarily with the production of winged, or alate, forms when two variables were involved, namely, the stage of maturity of the plant at the time of infestation, and the size of the initial aphid population. Plants in four stages of growth were employed—very young, young, early-flower, and mature; and the initial infestation consisted of 1, 10, and 100 apterous adults on equal numbers of plants of each stage. From about 2 weeks after colonization, at frequent intervals until death, each plant was examined for winged aphids.

The fewest alate forms developed on the very young plants, principally because these plants were too small to support the initial population. When the original infestation consisted of 1 aphid per plant there was no real difference in the number of alate forms developed on plants of the other 3 stages of growth; but when it consisted of 10 aphids per plant, the number developing on young plants was larger than on either the early-flower or the mature plants; and when the infestation consisted of 100 aphids the number of alate forms developing on early-flower plants was almost significantly greater than on young plants.

Among plants of the same stage of maturity, the number of alate forms recovered from young plants was larger when the initial population was 10 aphids per plant than when it was 1 or 100. Larger numbers of alate forms were recovered from early-flower plants originally populated with 100 per plant than from those populated with 1.

The differences in average numbers recovered from mature plants of each subclass were not significant. In general, the time when production began, the rate of production, and the time of peak production of alate forms appeared to be associated negatively with an increasing order in plant size and positively with an increasing order in size of initial aphid population.

It appears probable that the principal factors influencing the production of winged forms in this experiment were size of plants, intensity of aphid infestation, and condition of the plants resulting from the uniform watering procedure employed. Irrespective of the probable factors involved, however, it is evident that wild rutabaga is an important host of the green peach aphid and that it may constitute a serious source of infestation if permitted to grow in or near potato fields.

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THE ASSOCIATION OF HYLURGOPINUS RUFIPES WITH THE DUTCH ELM DISEASE PATHOGEN[1]

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INTRODUCTION

There seems to be general agreement that in the United States the bark beetle *Scolytus multistriatus* (Marsham) is the principal and most effective carrier of the Dutch elm disease pathogen, *Ceratostomella ulmi* (Schwarz) Buis. The disease occurs sporadically, however, in areas of New York State where this species is not known. The identification of the disseminating and inoculating agent or agents in such areas is the objective of the studies described herein.

Most of the field observations reported in this paper were made northeast of the City of Binghamton, N. Y., in Broome and Chenango Counties, where the European elm bark beetle *Scolytus multistriatus* has not been found, but where the native elm bark beetle *Hylurgopinus rufipes* (Eich.) is prevalent. Laboratory studies correlated with the field work were made chiefly with material collected in this area.

REVIEW OF LITERATURE

Britton (4)[3] and Clinton and McCormick (7) called attention to the fact that *Hylurgopinus rufipes* might transmit the Dutch elm disease to healthy trees. They isolated *Ceratostomella ulmi* from beetles of this species taken from the bark of a diseased tree in the field, and they found that when beetles from diseased trees were confined in test tubes with twigs cut from healthy elm trees the beetles chewed the bark and the fungus fruited on the twigs. These workers observed that *Scolytus multistriatus* was not present at Old Lyme, Conn., but that *H. rufipes* was abundant on a diseased tree found there in 1934. Britton stated that *H. rufipes* may make feeding injuries on elm twigs. Kaston and Riggs (*13*) expanded these observations and suggested that the subsequent spread of the disease at Old Lyme probably was due to feeding by this species. They reported that it made feeding tunnels in the bark on branches as small as 1 inch in diameter, or

[1] Received for publication November 1, 1946.

[2] Many persons assisted with the work or made helpful suggestions. Especially substantial contributions were made by Dr. W. Howard Rankin, Anthony J. Osekosky, and B. H. Hodge of the Bureau of Plant Industry, New York State Department of Agriculture and Markets, and by Dr. Seth Pope of Cornell University. The Boyce Thompson Institute for Plant Research made available facilities for the laboratory work.

[3] Italic numbers in parentheses refer to Literature Cited, p. 183.

even smaller, but they did not observe injury to the wood. Becker (*2*, *3*) previously had reported bark tunnels on branches of that size similar to the winter tunnels in which he had occasionally observed injury to the wood.

Collins (*8*) reported an experiment in which 1 out of 11 elm trees became infected the year after adults of *Hylurgopinus rufipes* were confined on the trunks in the fall. Hagmann [4] confined fungus-bearing beetles on trees 1 to 4 inches in diameter during May and June and found infection from feeding injuries on the trunk near the origin of the first branch, or on the branches themselves, in 3 of 19 cages.[5] Collins stated that the hibernating adults, by boring into the tree in the spring, make contact with the wood, but that this is done before new wood vessels are produced and infection would not be expected at that time. He believes that adults arising later in the spring from overwintering larvae would make similar injuries to the wood after new vessels are present and that infection would then be expected. This apparent relation of time of inoculation to probability of infection seems to be supported by inoculation experiments (*6*, *15*).

Several workers have observed that the adults of *Hylurgopinus rufipes* may feed on small branches as well as large soon after they emerge, but there is a difference of opinion regarding the relative numbers of the beetles that overwinter as larvae and as hibernating adults (*12*, *14*), and therefore there is some question as to just when most of the feeding occurs.

Collins et al. (*9*) found that after emerging from diseased trees *Hylurgopinus rufipes* may carry the fungus pathogen into uninfected dead or dying elm material when making maternal galleries. Jones and Moses (*11*) isolated the fungus from substantial numbers of adults collected as they were attracted to felled healthy elm trees at different locations in New Jersey and in the Hudson River Valley in New York. The writers made studies, not reported in detail, similar to those of Collins et al. and to those of Jones and Moses, with similar results.

CULTURE STUDIES

EXPERIMENTAL PROCEDURE

Elm logs infested with the beetles were cut from diseased trees, or uninfested branches were cut from diseased trees and exposed to beetle attack in the field, and then placed in cages out of doors. The emerging adults were collected and isolation trials made for *Ceratostomella ulmi*. Cultures were made aseptically by crushing the beetles in water in petri dishes and mixing the macerated tissue with melted potato-dextrose agar.

In a similar manner, naturally infested material apparently not killed by the Dutch elm disease (such as cut wood, broken branches, and branches or trees dying because of poor environmental condi-

[4] HAGMANN, LYLE E. FEEDING HABITS AND RELATED ACTIVITIES OF THE TWO ELM SCOLYTIDS, SCOLYTUS MULTISTRIATUS (MARSHAM) AND HYLURGOPINUS RUFIPES (EICHHOFF) WITH REFERENCE TO THE SPREAD OF THE DUTCH ELM DISEASE PATHOGEN CERATOSTOMELLA ULMI (SCHWARZ) BUISMAN. 1945. [Unpublished doctor's thesis. Copy on file Cornell University library, Ithaca, N. Y.]

[5] In other work (unpublished) done at the Cornell University Dutch elm disease laboratory at Yonkers, N. Y., infection was obtained in a similar way on small potted trees.

tions) was collected in the field, placed in cages, and the emerging adults cultured.

For a more rapid method of determining the presence or absence of the fungus on beetles in the field, callow adults and pupae were collected in the field and cultured aseptically. These were obtained from cut wood and naturally occurring dead wood which apparently had not been killed by the Dutch elm disease.

A study was also made to determine whether the fungus would persist on hibernating adults. Beetles were taken from cages containing logs from which adults bearing the fungus had been cultured, placed on cut healthy logs late in the fall, and stored over the winter in small screen cages out of doors. The following spring the overwintered live beetles were dug out of the logs and cultured. Hibernating adults collected in the field during the winter and spring were also cultured.

BEETLES EMERGING FROM WOOD CUT FROM DISEASED TREES

In 1937, wood not infested with beetles was cut from diseased trees and stored at 5° C. In the spring of 1938, it was exposed in the field to beetle entry for egg laying at Armonk, Westchester County, N. Y. and on July 8 was placed in emergence cages out of doors at Yonkers.

On July 13, 1940, material infested with *Hylurgopinus rufipes* was cut from a diseased tree at Sanford, Broome County, N. Y., and on August 3 it was placed in an emergence cage out of doors. The results of cultures made from *H. rufipes* adults that emerged from the two sets of material are shown in table 1.

TABLE 1.—*Results of cultures made from Hylurgopinus rufipes adults emerging from wood from diseased trees infested in the field*

Source of wood	Period of collection of beetles for culturing	Beetles		
		Total	With *C. ulmi*	
			Number	Percent
Diseased wood infested at Armonk, N. Y.	July 26–Aug. 7, 1938	261	32	12.3
Infested wood from diseased tree at Sanford, N. Y.	Aug. 8–Aug. 16, 1940	38	30	78.9

BEETLES EMERGING FROM NATURALLY INFESTED MATERIAL FROM NONDISEASED TREES

In 1940, in connection with a survey to determine the incidence of *Ceratostomella ulmi* on *Scolytus multistriatus* emerging from naturally infested material in Westchester County during the late summer, *Hylurgopinus rufipes* also was obtained from many of the samples. Eight to 451 (usually about 150) adults of the latter species were cultured from each of 41 wood collections, which consisted of 3 logs each 4 to 5 inches in diameter and about 16 inches long. The fungus was obtained from beetles from 7 of the 41 stations.

During the same summer similar survey material was collected in the disease area in Broome and Chenango Counties. The fungus was obtained from *Hylurgopinus rufipes* adults emerging from 4 of the 11 collections.

ADULTS AND PUPAE COLLECTED IN THE FIELD

In 1941, collections from infested material were made at various stations in Broome and Chenango Counties. Adults or pupae, or both, were taken as they were available at the time the station was visited. Table 2 gives the results of cultures made from beetles collected within or at the edge of the area of disease incidence as known in 1941. Beetles carrying the fungus were obtained at 7 of the 14 stations. At these 7 stations 98 adults and pupae of a total of 1,297 gave positive cultures.

TABLE 2.—*Results of cultures made from Hylurgopinus rufipes collected from infested material in the field in 1941*

Site No.	Number of bark samples	Total area of bark sampled (square feet)	Beetles cultures (adults and pupae)			Number bark samples from which beetles yielding positive cultures emerged
			Total	With *C. ulmi*		
				Number	Percent	
1	4	3.6	200	41	20.5	4
2	4	10.2	203	14	6.9	4
3	4	4.0	200	6	3.0	2
4	4	2.7	199	0	0	0
5	4	.7	198	2	1.0	1
6	1	6.0	56	0	0	0
7	1	6.0	96	14	14.6	1
8	4	18.0	201	0	0	0
9	4	15.9	200	2	1.0	1
10	4	7.9	200	19	9.5	2
11	4	4.5	212	0	0	0
12	1	5.0	99	0	0	0
13	4	7.5	201	0	0	0
14	1	10.0	24	0	0	0

PERSISTANCE OF FUNGUS ON HIBERNATING ADULTS

From September 30 to November 10, 1936, beetles emerging from material from diseased trees (cages Rh 29 and Rh 30) were introduced into cages out of doors (cages Rh 36 and Rh 37) containing wood freshly cut from healthy elm trees in which these beetles could hibernate. On April 15, 1937, beetles that had lived through the winter were collected from this wood for culturing. At that time they were just beginning to make maternal galleries. The dead beetles were not cultured. The data gathered in this experiment are presented in table 3.

TABLE 3.—*Comparison of results of cultures of adults of Hylurgopinus rufipes emerging from diseased wood in the fall and from hibernation in the spring*

Source of beetles (emergence cage No.)	Results of culturing beetles from original source material, fall of 1936			Source of beetles (hibernation cage No.)	Number beetles introduced, fall 1936	Results of culturing beetles from hibernation cages, spring of 1937		
	Number cultured	Number with C. ulmi	Percent with C. ulmi			Number cultured	Number with *C. ulmi*	Percent with *C. ulmi*
Rh 29	129	73	56.6	Rh 36	193	19	11	57.9
Rh 30	169	62	36.7	Rh 37	130	11	7	63.6

At intervals during the winter of 1940–41 and the spring of 1941 beetles were collected from their hibernation galleries in the field and cultured. All collections were made within the known area of disease incidence in Broome and Chenango Counties from the trunks and large branches of healthy trees. The time of year each collection was made may be roughly determined from the date on which the cultures were made. This was within 30 days of the time of collection of the beetles. Information on these experiments is given in table 4.

TABLE 4.—*Cultures of hibernating adults of Hylurgopinus rufipes collected from healthy trees in the field*

Month of culturing	Number of stations	Number of beetles	Number of cultures	Number of cultures with *C. ulmi*
November 1940	1	43	43	0
December 1940	7	188	188	4
February 1941	9	110	110	1
May 1941	8	146	36	2
June 1941	8	213	67	0

FIELD OBSERVATIONS IN THE UPSTATE AREA OF DISEASE INCIDENCE

Following the discovery in 1939 of the Dutch elm disease in Broome County, N. Y., by scouts of the United States Department of Agriculture, detailed observations were made on many of the infected trees found in that area. These studies were similar to those made in the Hudson River Valley (10). Since *Scolytus multistriatus* is not known to occur in the Broome County area, and it has been shown that *Hylurgopinus rufipes* in that area carries the fungus and can inoculate healthy trees, it is probable that the latter species inoculated all the diseased trees that were observed.

In 1940, 56 diseased trees were observed at 30 sites. In 34 of these trees it was estimated that at least 10 percent of the branches were dead and had harbored or were still harboring *Hylurgopinus rufipes*. In addition, unsuccessful maternal galleries had been attempted in at least 4 other trees.

In 1941, 30 diseased trees were observed at 22 sites. In 15 of the trees, at least 10 percent of the wood was dead.

At each site an attempt was made to determine the year when each tree first became infected and the nearest source of beetles. This particular observation was independent of the subsequent history of the diseased trees. That is, the beetle emergence referred to here occurred prior to infection of the trees.

Table 5 lists the types of wood which contained the observed beetle sources and the distances from the diseased trees to the nearest probable beetle sources. In some instances no source was found. The observed emergence usually, but not necessarily always, occurred at a time when the beetles could have made the specific inoculation involved. In most of the diseased trees cited as being their own sources of beetles, the branches from which beetles had emerged

were killed by unfavorable soil conditions or other environmental factors. Such trees are included among those listed as being within 100 feet of the source of beetles.

TABLE 5.—*Types of beetle sources and number of diseased trees associated with them, and distances from beetle sources to diseased trees*

Type of beetle source and distance from source to diseased trees	Number of diseased trees associated with beetle sources		
	1940	1941	Total
Types of beetle source:			
Branch of same diseased tree [1]	24	5	29
Other diseased trees [2]	8	2	10
Dead trees or dead branches in other trees	21	10	31
Broken-off branches or logs	0	5	5
Source unknown	3	8	11
Distances from beetle source to diseased trees:			
100 feet or less	36	15	51
Between 100 and 250 feet	12	7	19
Between 250 and 500 feet	5	0	5
Unknown	3	8	11
Total trees observed	56	30	86

[1] Beetles emerged before tree became infected.
[2] Only sources of beetles observed that are believed to have been diseased branches. All others listed in this table were in material not killed by the Dutch elm disease.

Feeding tunnels as described by Kaston (*12*) and others were observed on many of these trees. Many tunnels reached and injured the wood, especially on the smaller branches, ¾ inch to 4 inches in diameter, where the bark was thin. Injury to the wood was also observed apparently arising from hibernation galleries as described by Becker (*2*). The appearance of the injury and adjacent recent extension to the hibernation tunnel when seen in the spring usually indicated that wood injury was made at that time.

A rather high proportion of the diseased trees contained live colonies of *Hylurgopinus rufipes*. It is suggested that many such trees were inoculated by the beetles in connection with oviposition in portions which were still in a state of suitable physiological activity to permit infection at the time of the actual or attempted oviposition. There are no published data demonstrating that infection results from tunnels made for egg laying, but it has been shown that trial entries by *Scolytus multistriatus* may result in effective inoculations.[6] On trees with completed galleries of *H. rufipes*, trial entries or feeding tunnels which injured the wood were sometimes found in adjacent, somewhat more vigorous branches.

The incidence of diseased trees in this area was very low, an average of one in 5 square miles in 1940 and one in 10 square miles in 1941. Frequently, the disease attacked the weakest tree in a group. That is, trees receptive to beetle entrance became diseased first. Later, perhaps the following year as a rule, more vigorous trees in the neighborhood became diseased. Probably, in diseased tree associations of this kind the first inoculations were made by trial entries for egg laying,

[6] Unpublished work at Cornell University Dutch elm disease laboratory at Yonkers, N. Y.

as in observations reported by Collins (*8*). Subsequently, the bettle population was built up to such an extent that the relatively small percentage making injuries to the wood in feeding activities was sufficient to make a few effective inoculations.

DISCUSSION

The following discussion is an attempt to interpret the results of these studies, and the low incidence of the Dutch elm disease in the area northeast of Windsor, N. Y., where the bark beetle species *Scolytus multistriatus* is absent but *Hylurgopinus rufipes* is present, in the light of published information on the life history and habits of the latter species. Since *Ceratostomella ulmi* was obtained from *H. rufipes* adults taken from cut or broken elm branches originating in nondiseased trees, the breeding activities of this species is probably a means of introducing the fungus into new areas. The abundance of the fungus in such material in the field was substantially as great as that found by Collins et al. (*10*) associated with *S. multistriatus;* that is, it was obtained from beetles from similar percentages of wood samples, and the number of fungus colonies per beetle was about the same.

Probably the time when inoculations are most likely to cause destructive infections is early in the spring, but not until after new xylem vessels are produced. Any one of three methods of inoculation by *Hylurgopinus rufipes* might be operative at this time. Many of the wood injuries resulting from extensions of hibernating tunnels and from attempted entries by overwintered adults may be made before new xylem vessels are produced and such injuries would not be likely to result in infection. However, according to Kaston (*12*), some of these beetles do not leave their hibernation tunnels until late May or June, and infection should result from inoculations made by them. Feeding by beetles that arise from overwintering larvae has been shown to cause infections. There is also the possibility that effective inoculations may be made in the middle of the summer or later by this species. The work of Banfield (*1*) indicates that the movement of the fungus spores when introduced into trunks or large branches during the latter part of the growing season is slower and less extensive than earlier in the season. But the movement is extensive enough to make it appear plausible that inoculations made either by trial entry or by feeding by *Hylurgopinus rufipes* late in the season might be effective. Furthermore, since this species feeds in larger branches, inoculations made in the summer would be more likely to result in infection than would those made at the same time by the feeding of *Scolytus multistriatus* in the small twigs. One of the writers (Tyler) [7] found that hypodermic inoculations on the trunks late in the season were more successful than those made on the twigs at the same time.

Regardless of the several possible methods by which *Hylurgopinus rufipes* may make inoculations, the number of known infections in areas in New York State where it is abundant and *Scolytus multistriatus* is absent are relatively few (*5*). This fact is undoubtedly associated with two well-defined relationships, namely, that (1) egg-laying trial

[7] Unpublished data.

tunnels apparently are usually made in branches with insufficient vigor for the fungus to invade the live parts of the trees, and (2) wood injuries made in connection with hibernation and feeding activities are apparently not sufficiently frequent, or are made at the wrong time of the year, to account for many inoculations. Detailed observations by the writers on nearly 100 diseased trees and more casual observations over a period of several years on many more have failed to reveal abundant injuries of any of these types on sound branches. When compared with the deep-feeding injuries to the twigs made by *S. multistriatus* they would be considered as rare.

SUMMARY

The bark beetle *Hylurgopinus rufipes* may carry the Dutch elm disease pathogen when emerging from infested wood originating in diseased trees, cut elm wood, or dead branches and trees. The fungus may survive the winter on hibernating adults. It seems to be adapted to a congenial association with this native beetle in dead elm wood.

Field observations on the incidence of diseased trees in New York State indicate that *Hylurgopinus rufipes* has been very much less effective as an inoculating agent in the area studied than *Scolytus multistriatus* in the Hudson River Valley. One reason for this is the fact that *H. rufipes* is much less likely than *S. multistriatus* to make injuries that reach to and into the wood of sound trees and branches.

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